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RESEARCH ARTICLE

Description of immature stages and natural history of *Stigmella schinivora* (Lepidoptera: Nepticulidae), a leaf-miner associated with the Brazilian peppertree

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ABSTRACT. *Stigmella schinivora* van Nieukerken, 2016 was described from Cataratas de Iguazú, Misiones, Argentina, based on adults reared from *Schinus terebinthifolius* Raddi (Anacardiaceae) leaf mines. The aim of this study is to describe for the first time the external morphology of the immature stages of *S. schinivora* with the aid of light and scanning electron microscopy, based on mines collected on the same host plant, but in Laranjeiras do Sul, Paraná, Brazil. Data on natural history, including histology of the mines, are also provided. The larva passes through four instars, all endophytic, having chewing mouth parts and feeding on the palisade parenchyma. The first three instars are apodous and have a subcylindrical body, bearing only one pair of setae on the tenth abdominal segment; the fourth instar is eruciform, with well-developed ambulatory calli on thorax and abdomen and setae on all tagmata. A serpentine mine is constructed on the adaxial surface, progressively increasing in width during larval development. With the exception of the widened, terminal section, the mine is left filled with larval feces. The fully developed larva of last instar exits through a slit made at the distal end of the mine, building a silk cocoon on the leaf abaxial surface where pupation occurs. This is the first record of *S. schinivora* from Brazil, which was only known from the type locality in Argentina.

KEY WORDS. Atlantic forest, leaf-mining moths, microlepidoptera, nepticulids, *Schinus terebinthifolius*.

INTRODUCTION

The Nepticulidae is one of the most ancient lineages of Lepidoptera, with a global distribution and approximately 884 described species that are divided into 29 genera (van Nieukerken et al. 2016a, van Nieukerken 2018). They are among the smallest extant lepidopterans, having predominantly leaf miner habits and being associated with several plant families (Braun 1917, van Nieukerken et al. 2016a). Their minute size and the scarcity of the material available in collections has led their taxonomy to be mainly based on the morphology of adults, the immature stages rarely being taken into account (e.g., van Nieukerken et al. 2004). Studies that include the general appearance of their mines are not uncommon (e.g., Braun 1917, Stonis et al. 2013, 2014, van Nieukerken et al. 2016b), but specialization of larval feeding on tissues, if any, is largely unknown.

The worldwide distributed genus *Stigmella* Schrank, 1802 currently with ca. 420 species, is the largest genus of Nepticulidae (van Nieukerken 2018). Species identification in this genus can be difficult, since species complexes are common whose adults have similarities in external appearance; in these cases, only subtle differences in the genitalia morphology can be detected among species (Stonis and Remeikis 2016). There are at least 61 species of *Stigmella* recognized for the Neotropical Region (van Nieukerken et al. 2016a); however, this genus is still little studied in this region; thus, this number may not reflect its real diversity in the Neotropics (Puplesis and Robinson 2000, Šimkevičiūtė et al. 2009, Stonis et al. 2014, van Nieukerken et al. 2016a, Stonis and Remeikis 2017). This aspect is even more relevant in Brazil, where there are no records of *Stigmella* yet. This is unexpected since this is a megadiverse country, including biomes such as the Atlantic Forest, known for the great diversity of plants and animals and high endemism indexes (Myers et al. 2000).

Stigmella schinivora van Nieukerken, 2016 was recently described as a leaf miner of the Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae) from the region of Misiones, Argentina (van Nieukerken et al. 2016b). Its description relied on morphology of the male and female genitalia. In the present study, using material collected in southwest Paraná state, Brazil, we present a detailed description of the immature stages of *S. schinivora*, based on light and scanning electron microscopy. We also provide additional information about its natural history, including the histology of its mines on leaves of *S. terebinthifolius*.

MATERIAL AND METHODS

Specimens used in this study came from leaf mines of *S. terebinthifolius* collected in Laranjeiras do Sul municipality, Paraná, Brazil, in 2016 and 2017. They were brought to the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Zoology Department of Federal University of Rio Grande do Sul (UFRGS), Porto Alegre city, and then they were either dissected or kept at room temperature in plastic pots containing moistened cotton for emergence of adults. The adults obtained in the laboratory were identified as *S. schinivora* based on comparison with original descriptions and illustrations of the adult stage, including female and male genitalia, provided by van Nieukerken et al. (2016b).

Adults were pinned and dried. Immature stages were fixed in Dietrich's fluid and preserved in 75% ethanol. For descriptions of the gross morphology, the specimens were cleared in a 10% potassium hydroxide (KOH) solution and slide-mounted in either glycerin jelly or Canada balsam. Observations were performed with the aid of a Leica M125 stereomicroscope, and measurements were performed using an attached ocular micrometer (precision = 0.01 mm). Structures selected to be drawn were previously photographed with a Sony Cyber-shot DSC-H10 digital camera attached to the stereomicroscope, and also by using a Nikon AZ 100M stereomicroscope. Vectorized line drawings were then made with the software Corel Photo-Paint X7, using the corresponding digitalized images as a guide. At least five specimens were used for the descriptions of each morphotype.

For scanning electron microscope analyses, additional specimens were dehydrated in a Bal-tec CPD030 critical-point dryer, mounted with double-sided tape on metal stubs and coated with gold in a Bal-tec SCD050 sputter coater. They were examined and photographed in a JEOL JSM6060 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of UFRGS.

For plant anatomical descriptions, field-collected leaf parts of *S. terebinthifolius* containing mines of *S. schinivora* were preserved in Dietrich's fluid. Leaf parts containing the different larval instar morphotypes were selected under a stereomicroscope, and freehand cross sections were cut with a razor blade. They were then stained for five seconds with safranin and photographed with a Nikon AZ 100M stereomicroscope.

Vouchers of specimens used in this study were deposited in the insect collection of the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Zoology Department (UFRGS), as follows (all coming from *S. terebinthifolius* leaf-mines collected by the senior author at Laranjeiras do Sul, Paraná, Brazil): 16-23.VII.2016, pinned, dried adults, two females (LMCI 309-10 and 11, with genitalia on slides GRPM 50-151 and 152, respectively), two males (LMCI 309-12 and 13, with genitalia on slides GRPM 50-153 and 154, respectively); 29.XI.2017, immature stages, fixed in Dietrich's fluid, preserved in 70% ethanol, 3 first instar larvae (LMCI 323-2), 3 second instar larvae (LMCI 323-3), 4 third instar larvae (LMCI 323-4), 8 fourth instar larvae (LMCI 323-5) and 3 pupae (LMCI 323-7).

RESULTS

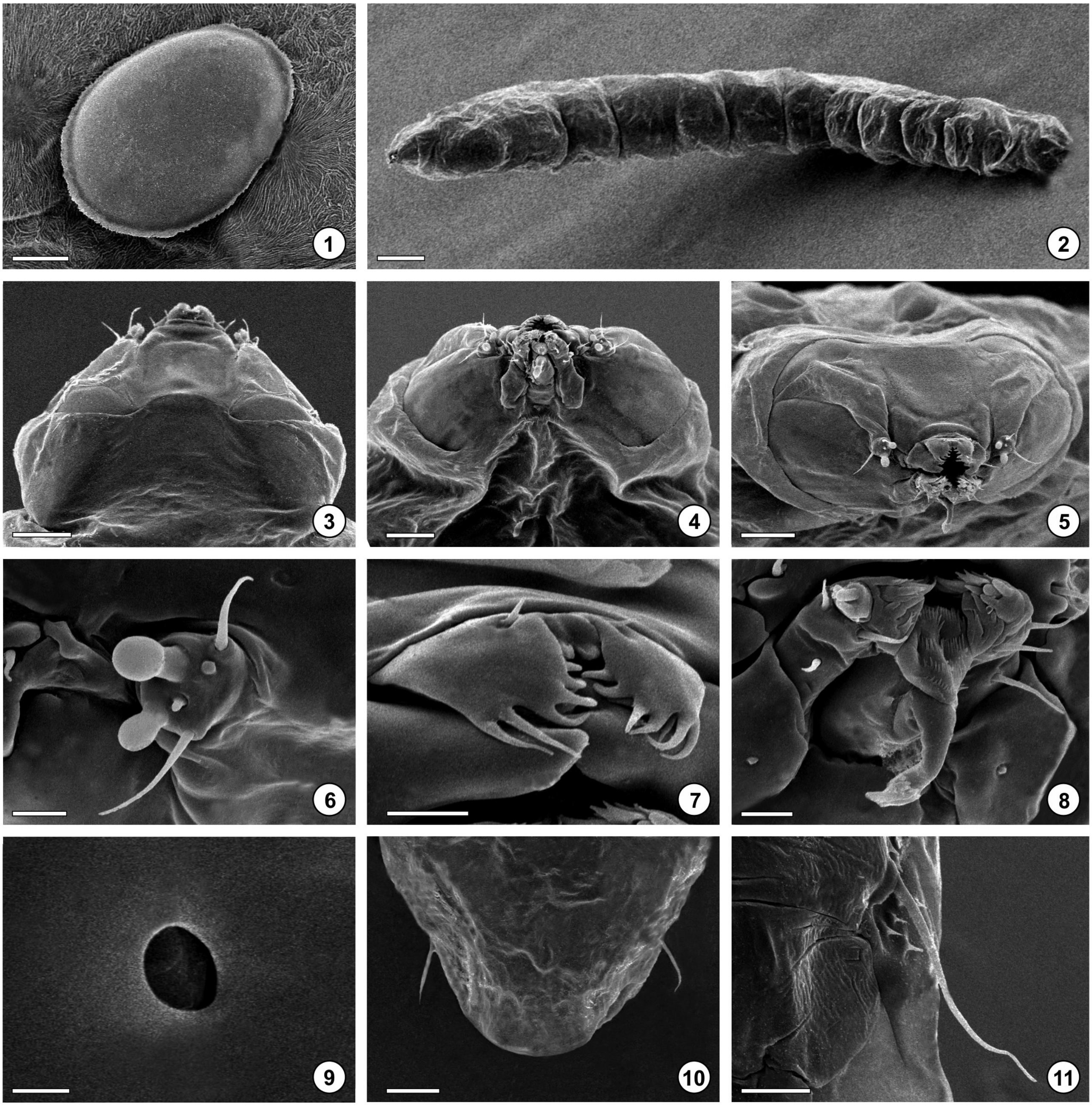
Egg. Flat and oval, firmly adhered to the leaf surface by a glistening substance (Figs 1, 44); average diameter + standard deviation = 0.16 ± 0.002 mm, n = 5. It is covered by a solid, smooth, transparent layer, forming a cap; micropyles and aeropyles were not found.

Larva. Prognathous, with buccal apparatus of chewing type. There are four instars and two morphotypes; the first form corresponds to the first three instars and the second to the last instar. The first morphotype has a subcylindrical, smooth body, without specialized locomotor structures (Fig. 2). The second morphotype has well-developed calli on thorax and abdomen, and setae of variable sizes distributed throughout the body (Fig. 19). We could not find major morphological differences among instars of the first morphotype. However, they can be identified by their size, since corresponding head capsule widths do not overlap (Table 1). The following exponential growth equation was adjusted for the head capsule width: $y = 0.049e^{0.400x}$; n = 37; r = 0.99; $p < 0.0001$.

Penultimate instar. Except for the absence of stemmata, the head of the first morphotype is similar to that of the second one in general color, shape (Figs 3–5), antennae (Fig. 6) and mouth parts (Figs 4, 5, 7, 8), which are described in detail below. The same occurs in relation to thorax and abdomen, including spiracles (Fig. 9). No evident setae were found on the thorax or abdomen of the first morphotype, except for the tenth segment where a pair of conspicuous setae appear dorsolaterally (Figs 10, 11).

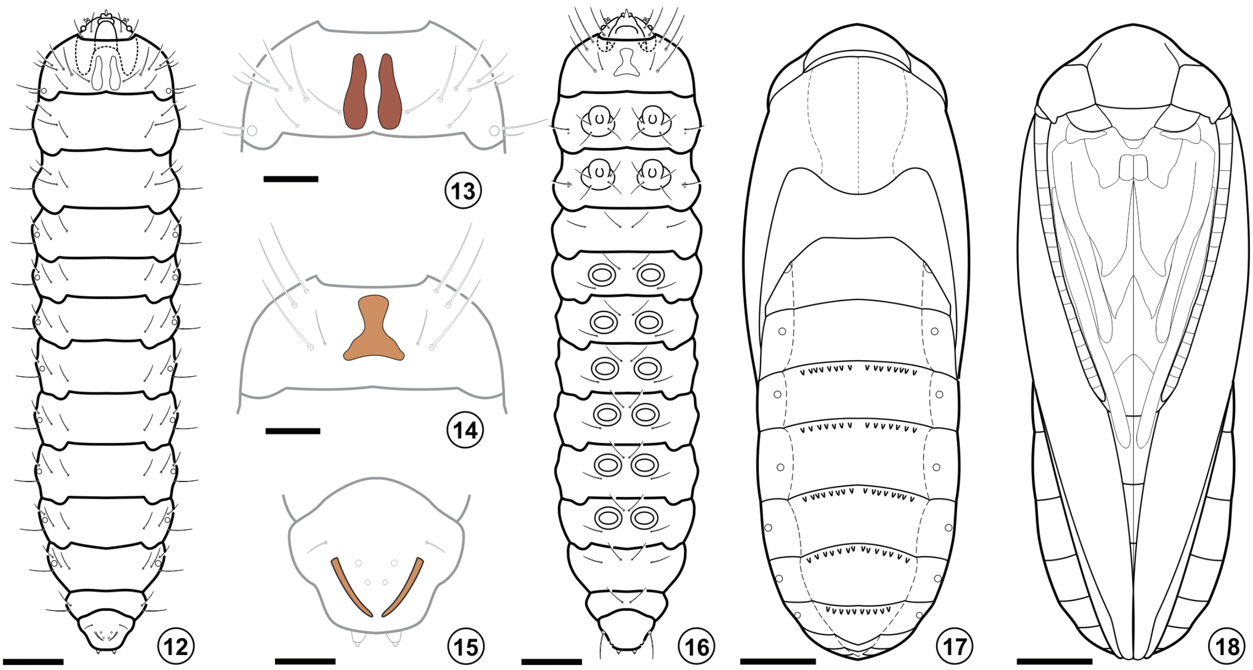
Table 1. Variation in size of head capsule width among instars of *Stigmella schinivora* reared on *Schinus terebinthifolius*.

Instar	N	Head capsule width (mm)		
		Mean ± standard error	Range	Growth rate
I	4	0.087 ± 0.003	0.084–0.095	–
II	5	0.116 ± 0.003	0.105–0.126	1.33
III	15	0.154 ± 0.002	0.147–0.168	1.33
IV	15	0.246 ± 0.002	0.231–0.263	1.60



Figures 1–11. Egg and third instar of *Stigmella schinivora* under scanning electron microscopy: (1) egg; (2) general view of larva, lateral; (3–5) head, under dorsal, ventral and anterior views, respectively; (6) antenna, anterior; (7) labrum, anteroventral; (8) labium, showing spinneret in detail, ventral; (9) spiracle of fourth abdominal segment, lateral; (10) last abdominal segment, dorsal; (11) seta of last abdominal segment in detail, dorsal. Scale bars: 50, 100, 20, 20, 20, 20, 5, 5, 1, 25, and 10 µm, respectively.

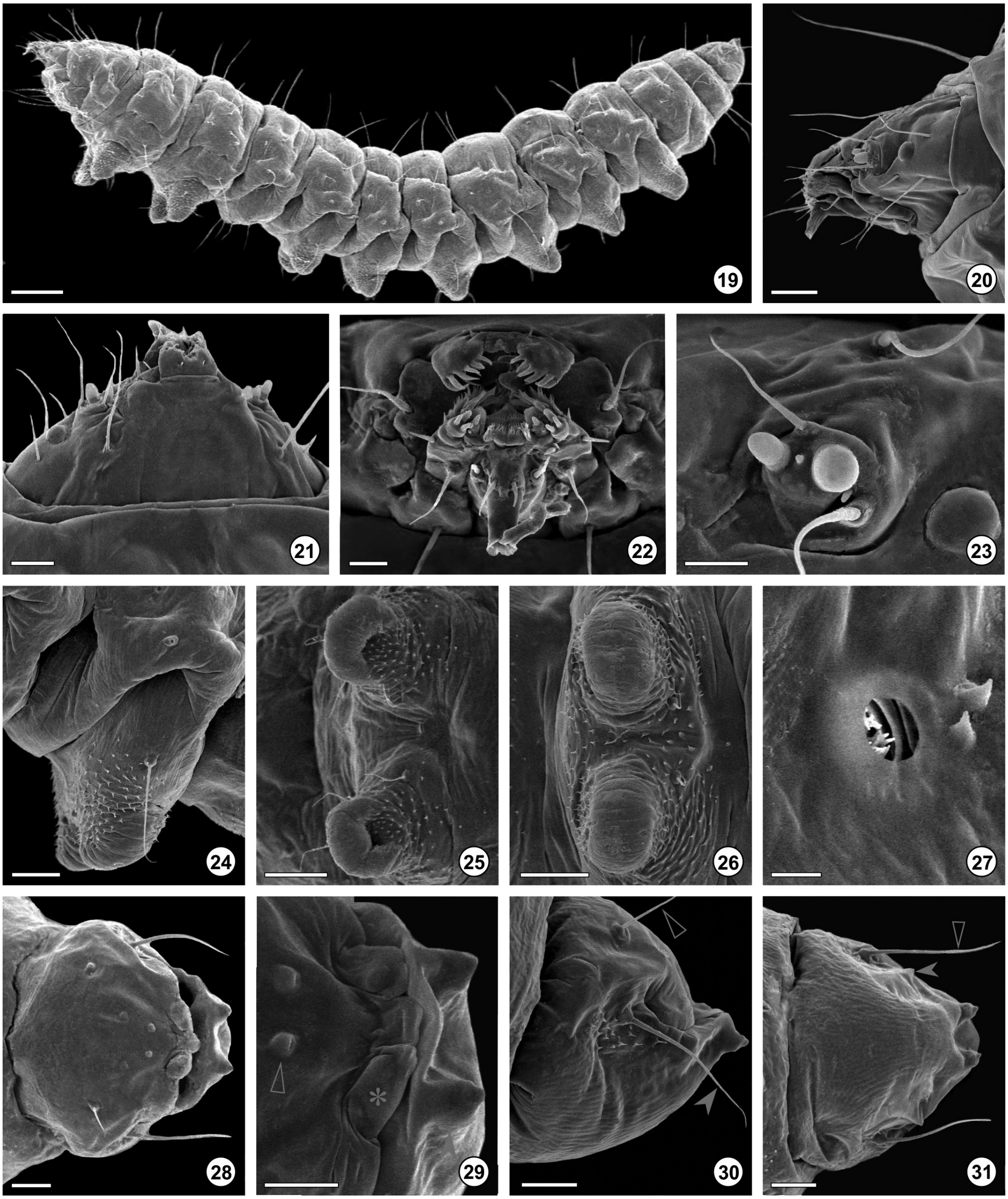
Last instar. Average length ± standard deviation = 1.42 ± 0.21 mm; n = 5. Head light brown, flattened dorsoventrally, partially concealed within the prothorax, with deep epicranial notch. Frontoclypeus rectangular, longer than wide. Labrum bilobed, with lobes having distal serrated edge, and bearing one pair of short setae mesally (Figs 21, 22); mandibles with well-developed cusps; one long seta on proximal base. Maxilla with well-developed galea and palpi. Labium with tubular spin-



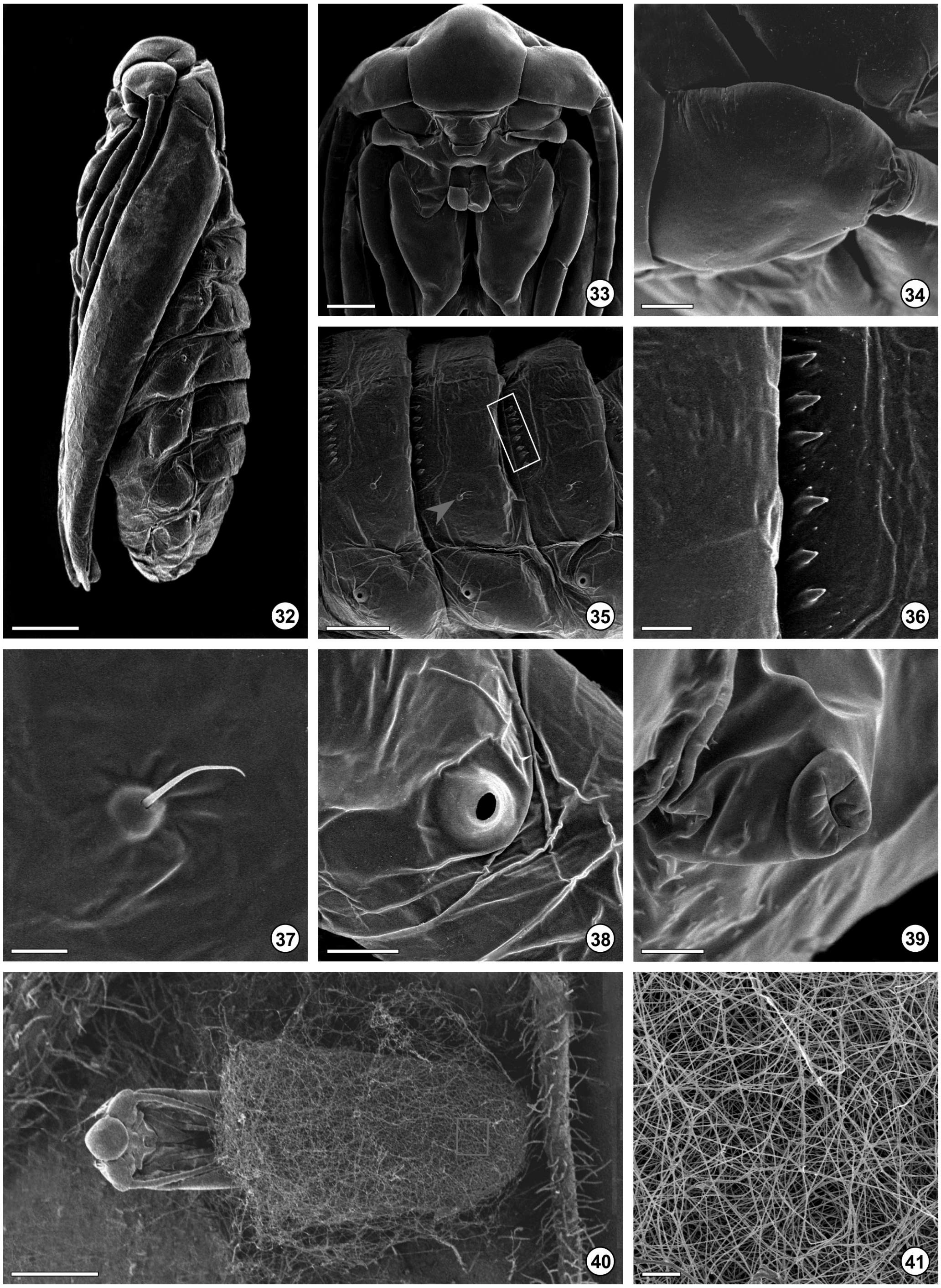
Figures 12–18. Last larval instar and pupal morphology of *Stigmella schinivora* under light microscopy: (12, 16) larva general, dorsal and ventral views, respectively; (13, 14) detail of tergal and sternal prothoracic plates seen through transparency, dorsal and ventral views, respectively; (15) anal rods of last abdominal segment, dorsal. (17, 18) pupa, dorsal and ventral, respectively. Scale bars: 300, 150, 150, 150, 300, 200, and 200 µm, respectively.

neret (Figs 20, 22), with a pair of setae on proximal base; labial palpi unisegmented, bearing a distal seta. Antenna unisegmented, with six apical sensilla; two minute in size, two stout and rounded, and two long and filiform (Fig. 23). A single, circular stemma, posterior to the antenna (Fig. 20). Thorax and abdomen cylindrical, creamy white in preserved material, bearing well-developed filiform setae. Thorax with integument smooth on T1 and sculptured with microtrichia ventrally in T2,3. T1 bearing a light brown shield on the tergum, divided into two elongated, meso-longitudinally arranged plates (Figs 12, 13); a light brown, cup-shaped plate on center of ventral prothoracic sternum (Figs 14, 16). A pair of lateral spiracles without elevated peritreme laterally on prothorax; legs and ambulatory calli absent. T2,3: Dorsal surface smooth; well-developed ambulatory calli ventrally, with the base wider than the transversally rounded apex, bearing an invagination on middle of the posterior wall (Figs 16, 24, 25). Each callus has the base sculptured with microtrichia and the distal edge smooth (Figs 24, 25). Abdominal segments similar in size from A1 to 8; A9 narrower; A10 smaller and subtriangular in shape. Integument mostly smooth on A1 and A10; partially sculptured with microtrichia dorsally on A3-8, laterally on A8-10 and ventrally on A2-9. Spiracles circular, without elevated peritreme (Fig. 27), laterally, from A1 to 8. Pairs of ambulatory calli present on A2-7, differing from the thoracic ones mainly by not having invagination (Fig. 26). A10 smooth, with a pair of light brown, longitudinally arranged, distally converging anal rods that are seen by transparency (Fig. 15); two pairs of triangular-shaped projections, one ventrolaterally (Fig. 31), the other on the distal edge of the segment (Figs 29, 31).

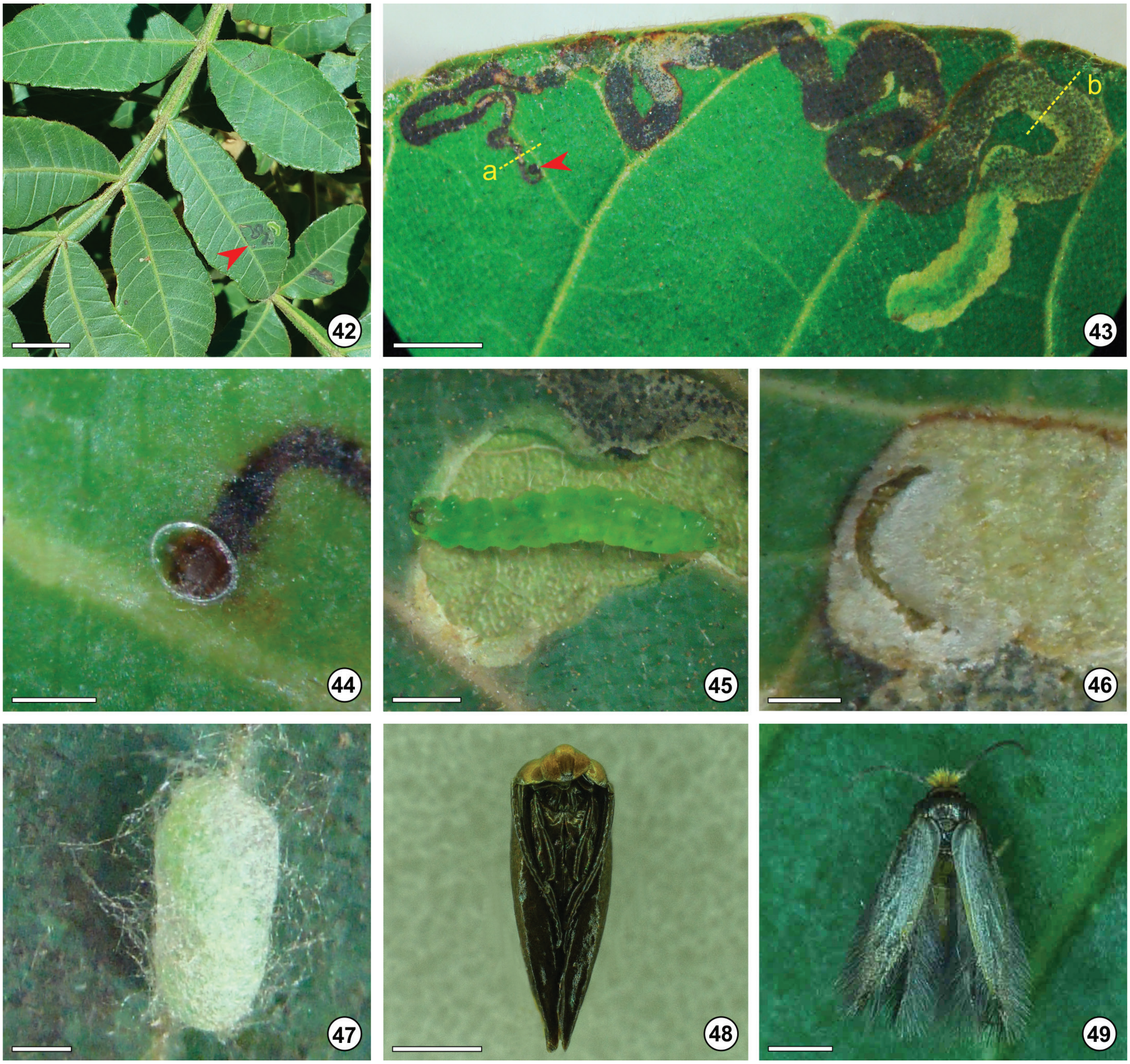
Chaetotaxy of the last instar larva. Head: Most of the setae are absent. Anterior group (A) and stemmatal group (S) unisetose. Substemmatal group (SS) bisetose. Thorax: T1 with thirteen pairs of setae. Dorsal group (D) bisetose; D1 near the lateral margin of the dorsal plate, D2 between D1 and spiracle. Extra dorsal group (XD) bisetose. Subdorsal group (SD) bisetose. Lateral group (L) trisetose; L1 and L3 ventral to spiracle, L3 between spiracle and L1, and L2 anterior to the spiracle, ventral to SD1. Subventral group (SV) trisetose; SV1 near the ventral plate, SV2 near the head capsule, and SV3 between SV1 and SV2. Ventral group (V) unisetose in the ventral plate margin. T2-3 with ten pairs of setae. Dorsal group (D) unisetose. Subdorsal group (SD) and lateral group (L) bisetose; L2 half of the length of L1. Subventral group (SV) trisetose; SV1 and SV3 in the callus, SV2 lateral to the callus. Ventral group (V) bisetose; V1 and V2 on the callus. Abdomen: A1–8 with six pairs of setae. Dorsal group (D) unisetose. Subdorsal group (SD) bisetose; SD1 between D2 and spiracle, SD2 near and anterior to spiracle. Lateral group (L) unisetose; L1 posteroventral to the spiracle. Subventral group (SV) unisetose. Ventral group (V) unisetose, near the ventral medial line. A9 with chaetotaxy similar to the



Figures 19–31. Last larval instar of *Stigmella schinivora* under scanning electron microscopy: (19) general view, lateral; (20, 21) head, lateral and dorsal views, respectively; (22) mouthparts, anterior; (23) antenna, anterior; (24, 25) mesothoracic ambulatory calli, lateral and ventral views, respectively; (26) ambulatory calli of sixth abdominal segment, ventral; (27) spiracle of eighth abdominal segment, lateral; (28) last abdominal segment, dorsal; (29) detail of tenth abdominal segment showing distal portion of anal rods (indicated by asterisk), posterior projections (indicated by closed arrow) and vestigial setae (indicated by open arrow), dorsal; (30) D2 and L1 setae, indicated by open and closed arrows, respectively, lateral; (31) last abdominal segment showing lateral projection (indicated by closed arrow) and L1 setae (indicated by open arrow). Scale bars: 200, 20, 150, 10, 10, 50, 50, 50, 5, 20, 20, 20, and 20 µm, respectively.



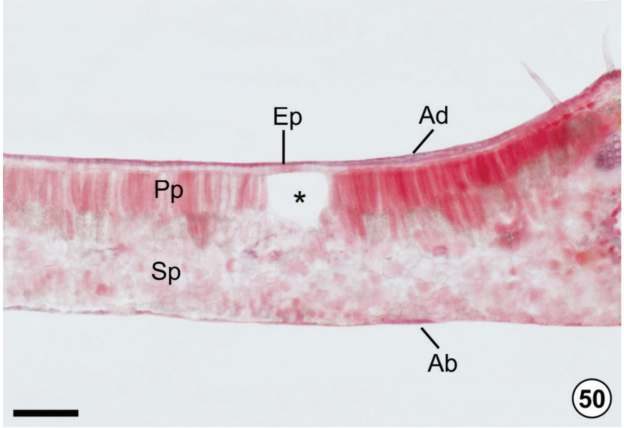
Figures 32–41. Pupa of *Stigmella schinivora* under scanning electron microscopy: (32) general view, lateral; (33) head and mouthparts, ventral; (34) eye-cap in detail, anteroventral; (35) third, fourth and fifth abdominal segments, laterodorsal; (36) detail of dorsal spines of fifth abdominal segment (indicated by rectangular area marked in Fig. 35), laterodorsal; (37) detail of fourth abdominal segment seta (indicated by arrow in Fig. 35), laterodorsal; (38) spiracles of third abdominal segment, laterodorsal; (39) spiracle (apparently closed) of eighth abdominal segment, lateral; (40) cocoon, with pupal exuvium extruded, ventral; (41) weaving pattern of the pupal cocoon surface (enlarged area marked by rectangle in Fig. 40). Scale bars: 200, 100, 40, 100, 30, 10, 20, 10, 500, and 50 µm, respectively.

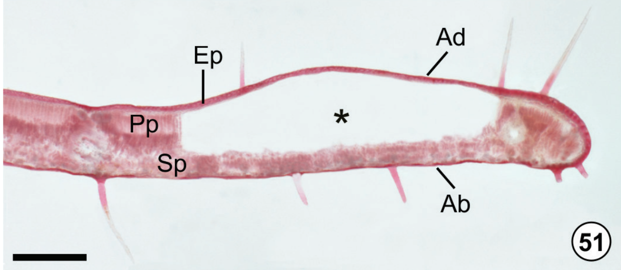


Figures 42–49. Natural history of *Stigmella schinivora* on *Schinus terebinthifolius*: (42) host-plant leaf bearing leaf mine on the adaxial surface of a foliole (indicated by closed arrow); (43) general view of leaf mine on foliole, showing last instar larva seen through transparence (arrow points to empty egg, and letters indicate position of histological sectioning (treated in Figs 50, 51); (44) empty egg chorium in detail; (45) dissected mine at the final portion, showing last instar larva; (46) exit hole, used by a last instar larva to leave the mine; (47) pupal cocoon adhered to abaxial leaf surface; (48) pupa in detail, after removal of the cocoon, ventral; (49) adult on the leaf, dorsal view. Scale bars: 10, 1, 0.2, 0.5, 0.15, 0.5, 0.5, and 0.5 mm, respectively.

anterior segments, SD and SV group absent. A10 with four pairs of setae. Dorsal group (D) unisetose (represented by D2); two pairs of apparently rudimentary setae (D1 and SD2) (Figs 15, 28, 29). Lateral group (L) unisetose, represented by L1 (Figs 12, 30, 31), longer than D2.

Pupa. Average length ± standard deviation = 1.56 ± 0.03 mm, n = 5. Partially exarate, with distal portion of the wings slightly distant from the abdomen (Fig. 32). Body brownish during early development (darkening later in ontogeny) and flattened dorsoventrally (average maximum width ± standard





Figures 50–51. Transverse histological sections of *Schinus terebinthifolius* leaf (indicated by dashed lines "a" and "b" in Fig. 43), showing the organization of *Stigmella schinivora* mine during larval ontogeny. (50) First instar, initial linear section of mine (position indicated by letter "a" in Fig. 43); (51) last instar, final section of mine (position indicated by letter "b" in Fig. 43). Asterisks indicate leaf mine. (Ab) Abaxial surface of epidermis, (Ad) adaxial surface of epidermis, (Ep) epidermis, (Pp) palisade parenchyma, (Sp) spongy parenchyma. Scale bars: 0.1 and 0.2 mm, respectively.

deviation = 0.52 ± 0.007 mm, n = 5). Head vertex dome-shaped (Fig. 33), without projections or setae; front and clypeus also smooth, subtrapezoidal; labrum subtriangular and narrow; maxillae well-developed, positioned ventrally to maxillary palpi that are cuneiform and projecting mesally beyond the eyes; labial palpi short, located in between the maxillae. Antenna not reaching the apex of the mesothoracic legs (Fig. 18); scape enlarged, partially covering the eye (Fig. 34). Pronotum as a narrow stripe dorsally (Fig. 17); forewings covering the mesal portion of abdominal segments in ventral view (Fig. 18); hindwings mostly concealed by the forewings; prothoracic and mesothoracic legs visible ventrally, extending respectively to second and fourth abdominal segments; metathoracic legs mostly covered by the wings, extending to distal limit of the abdomen. Abdominal segments A3-7 with rows of posteriorly directed spines, located dorsally on the anterior margin (Figs 17, 35, 36). A pair of dorsal setae, positioned laterally, in the segments T2,3 and A1-7 (Figs 35, 37). Abdominal spiracles with elevated peritreme, opened in A1-7 (Fig. 38), partially closed in A8 (Fig. 39). Cremaster absent.

Life history. The egg is usually laid near a lateral vein on the adaxial leaf surface (Fig. 43). After eclosion the first instar larva bores into the leaf and begins to feed on the parenchyma, filling the empty egg with feces (Fig. 44). The serpentine mine is small and narrow, slowly widening throughout larval ontogeny (Figs 42, 43). From the beginning to the end of the mine, the larva feeds only on the palisade parenchyma (Figs 50, 51), which is formed by two layers of overlapping cells in the leaves of *S. terebinthifolius* (Grisi et al. 2011). The mine is filled fully with feces, which gives it a characteristic blackish appearance (Fig. 43). After completion of development, the last instar larva opens a hole (Figs 45, 46) in distal section of the mine through which it leaves, searching for a pupation site on the abaxial surface either of the same or an adjacent leaf. Cocoon yellowish, cylindrical and silk-made (Figs 40, 41, 47), having flimsy threads, some on the external surface used for attachment to the substrate. It bears a slit on anterior edge through which the pupa projects itself to the outside prior to adult emergence, leaving the exuvium partly protruded from the cocoon (Figs 40, 48, 49).

Densities of *S. schinivora* are generally low in Laranjeiras do Sul populations of *S. terebinthifolius*, and in most cases only one mine occurs per leaf and foliole. Mines with mining larvae of *S. schinivora* were collected mostly during the spring. Apparently, more than one generation occurs per year, which should be further explored.

DISCUSSION

Morphology of nepticulid eggs is still controversial, and has not yet been the subject of any detailed study (Davis 1998). According to Johansson et al. (1990), they are covered by a smooth helmet-shaped egg case, supposedly formed by secretion coming from the female colleterial glands. However, Kobayashi (1996) mentioned the existence of a chorion and presence of micropyle canals on the surface of *Stigmella castanopsiella* (Kuroko, 1978). That of *S. schinivora* is covered by a dome-shaped cap, which can be easily pulled off by physical pressure, thus being detached freely from the remaining egg contents in preserved material. Furthermore, neither micropyles nor aeropyles were found. To better resolve this question, we suggest that oogenesis should be explored in detail for *S. schinivora*, to test whether or not a true chorion is formed in this species.

The four larval instars found here for *S. schinivora* follow the general pattern recorded for nepticulids in general (Johansson et al. 1990). Although barely mentioned in the recent literature, the existence of two larval morphotypes has been known for a long time in other nepticulids, for example *Enteucha acetosae* (Stainton, 1854) (Sich 1908, 1909) and *Trifurcula immundella* (Zeller, 1939) (Sich 1917). The scarcity of setae on early instars was mentioned by van Nieukerken (2007) for *Acalyptris* Meyrick, 1921. The absence of true thoracic legs and also of abdominal prolegs bearing chochets, but prominent thoracic and abdominal ambulatory calli instead follows the general pattern found for the last instar of nepticulids (Davis 1987). We associated the existence of these structures with a need for locomotion outside the mine in search for the place to spin the cocoon and pupate.

The prothoracic dorsal shield found in the last instar of *S. schinivora* is similar to that described for other species of *Stigmella* by Gustafsson (1985); however, the ventral prothoracic plate shows differences compared to those present in other congeneric species described by him. Thus, this structure may provide diagnosable taxonomic characters, and should be explored further regarding its use in identification of *Stigmella* species.

The dorsal sclerotized structures seen by transparency on the last abdominal segment of *S. schinivora* have received different names, such "brace rods" (Stehr 1987), "bar-like" (Gustafsson 1981) and "anal rods" (van Nieukerken 2007, van Nieukerken et al. 2011). We opted for anal rods, since given their position it is very likely that these structures are functionally related to the anus. van Nieukerken et al. (2011) reported that the anal rods may be important in the taxonomy of larvae belonging to *Acalyptris* which should also be further explored in *Stigmella*.

An interesting characteristic of the first morphotype of *S. schinivora* is the presence of a single pair of setae in the tenth abdominal segment. van Nieukerken (2007) reported the presence of similar setae, but in A8 (three pairs) in earlier instars in *Acalyptris*. The large number and size of setae present in the last instar in *S. schinivora* suggest that these structures may be important from a sensorial perspective when outside the mine prior pupation. They have probably not arisen in this instar in particular, but instead were lost in the previous ones in association with their endophytic habit.

The comparison of chaetotaxy in Nepticulidae, particularly in *Stigmella*, showed little variation, suggesting a conserved pattern. Compared to the chaetotaxy described by Gustafsson (1981) for *Stigmella auromarginella* (Richardson, 1890), *S. schinivora* has in T2-3 absence of L3; in A8 presence of L1 and absence of SV2; and in A9 presence of SV2. In comparison to the chaetotaxy described by Gustafsson (1985) for *Stigmella rhomboivora* Gustafsson, 1985, *S. schinivora* has in T2 absence of L3 and in A9 absence of SV1 and presence of L1. Gustafsson (1981) also states that SV3 is absent in *Stigmella plagicolella* (Stainton, 1854) and *Stigmella paradoxa* (Frey, 1858), whereas it is present in *S. schinivora*.

Two setae have been described in the literature for the last abdominal segment of nepticulid larvae (e.g., Gustafsson 1981, 1985), but they have not been named. The designation of D2 and L1 in this study were inferred by comparing locations of setae in previous abdominal segments. We presume the two pairs of vestigial setae found dorsally in the last abdominal segment of *S. schinivora* had not been noticed in previous studies due to their reduced size. They are herein tentatively nominated according to Hinton's system (Stehr 1987), and thus corresponding homologies should be explored further in comparison to other nepticulids.

We are not aware of scanning electron microscopy studies on the pupal morphology of Nepticulidae. The enlarged first antennal segment of *S. schinivora* stands out, associated with the eye cap in the adult, as well as the absence of any trace of a differentiated process on the head dorsum (= cocoon cutter) and a cremaster on the last abdominal segment. These absences are generally found in the family, as there is no need for the cocoon cutter and cremaster, since, as in *S. schinivora*, there is usually a slit anteriorly on nepticulid cocoons through which the pupa projects partially to the outside prior to adult emergence (van Nieukerken et al. 2004). Line drawings and description of the pupa of *S. plagicolella* provided by Patočka and Turčani (2005) are similar to those shown here for *S. schinivora*. However, these authors do not mention the existence of eight closed abdominal spiracles in *S. plagicolella*, which occurs in *S. schinivora*. Compared to other nepticulid genera such as *Trifurcula* Zeller, 1848 (van Nieukerken et al. 2004), *Roscidotoga* Hoare, 2000 (van Nieukerken et al. 2011) and *Acalyptris* (van Nieukerken 2007), differences are found in the arrangement of posteriorly directed abdominal spines in A3-7; they form only one row in the anterior margin in *S. schinivora*, contrary to what is found in these genera in which four to five lines of these spines can be found.

The leaf mine of *S. schinivora* is similar in general shape to congeneric species (e.g. van Nieukerken et al. 2006, Stonis et al. 2013, 2016, Stonis and Remeikis 2017) and to others described for different genera within the Nepticulidae (e.g. van Nieukerken et al. 2011), demonstrating a uniform pattern of the family, even though they may use different host plants. Unfortunately, we did not find other studies addressing histology of mines in Nepticulidae, which precludes comparison with results reported here. Pereira et al. (2017) demonstrated that the damage caused by the gracillariid *Leurocephala chilensis* Vargas & Moreira, 2016 to the leaves of a plant in the same genus, *Schinus molle* L., is different from that caused by *S. schinivora*. Most gracillariids show two different kinds of mandibles during development, which may be used initially for slicing and eating only the adaxial epidermis, as is the case of *L. chilensis*. Chewing mandibles, as in *S. schinivora*, appear only in latter ontogeny for that species, and are also used to eat the palisade parenchyma until the end of larval development. The different adaptations observed in these two species using closely related hostplants reflect different evolutionary patterns of the families in resource usage (Hering 1951, Menken et al. 2010, Doorenweerd et al. 2016).

Finally, it is important to emphasize that morphology of the immature stages in particular has been increasingly taken into account as an aid in species identification among leaf-miner moths, as for example among gracillariids (Davis and Wagner 2011, Kobayashi et al. 2013, Brito et al. 2017). Information on immature stage morphology is also a precondition for understanding interactions of these stages with host plants, particularly when damage on tissues and histology of the mines are explored in conjunction with ontogeny (e.g., Brito et al. 2012, 2013, Vargas et al. 2015, Pereira et al. 2017). Thus, our results not only clarify the morphology of *S. schinivora* immature stages, but also could be used as an integrative framework for characterizing and comparing variation of immature stage morphology and associated host-plant interactions among other nepticulids and beyond.

This is the first report of *S. schinivora* in Brazil, expanding its geographical distribution that was restricted to the type locality in Argentina. *Schinus terebinthifolius* is widely distributed in southern South America (see Davis et al. 2011), and thus the range of *S. schinivora* may be much broader, and should be further explored.

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